



**Fig S2** UPLC chromatograms showing the conversion of various steroid substrates by different recombinant S25DHs from *Stl. denitrificans* heterologously produced in *T. aromatica*. **A**, S25DH<sub>1</sub> (0.5 mg ml<sup>-1</sup>); **B**, S25DH<sub>2</sub> (0.5 mg ml<sup>-1</sup>); **C**, S25DH<sub>3</sub> (2 mg ml<sup>-1</sup>), and **D**, S25DH<sub>4</sub> (1 mg mL<sup>-1</sup>). Black lines: 0 min; blue lines: 30 min; red lines: 120 min; green lines: 360 min of incubation. The following substrates were added at 0.5 mM: cholest-4-en-3-one (1), vitamin D3 (VD3) (3), 7-dehydrocholesterol (5), campest-4-en-3-one (7) and sitost-4-en-3-one (9). They were converted to 25-OH-cholest-4-en-3-one (2), 25-OH-VD3 (4), 25-OH-7-dehydrocholesterol (6), 25-OH-campest-4-en-3-one (8) and 25-OH-sitost-4-en-3-one (10).